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EFFECT OF CONVENTIONAL AND ADVANCED COAL CONVERSION BY-PRODUCTS ON THE PULMONARY SYSTEM

by

Catherine Aranyi
Jeannie Bradof
IIT Research Institute
Life Sciences Research Division
Chicago, Illinois 60616

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Project Officer

Judith A. Graham
Inhalation Toxicology Branch
Health Effects Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

HEALTH EFFECTS RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
RESEARCH TRIANGLE PARK, NC 27711

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16. ABSTRACT

To evaluate the environmental impact of different energy technologies, fly ash samples collected from a coal-fired and from an oil-fired electric power plant were used in aerosol inhalation exposures of mice. The effects of multiple 3-h exposures to the fly ash particles at 2 and 1 mg/m^3 aerosol mass concentration and <0.5 um mass median aerodynamic diameter were evaluated in male and female mice by examining the changes in their pulmonary free cells, in their susceptibility to streptococcus infection, and in the bactericidal activity in their lungs to inhaled Klebsiella pneumoniae. Generally no consistent differences could be discovered in the effects of the exposures between the two sexes. However, in a combined evaluation of both sexes, more and greater significant changes relative to controls were observed in the experimental parameters after inhalation of the oil power plant fly ash than after exposure to the coal fly ash. Thus, the overall results of the study indicate that the pulmonary defense system of mice was more adversely affected by the oil-fired power plant fly ash, a true stack emission effluent, than by the coal fly ash collected by electrostatic precipitator, an in-plant control device.

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, nonionizing radiation, environmental carcinogenesis, and the toxicology of pesticides, as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for nonionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

The use of coal for power generation and for production of liquid and gaseous fuels is expected to increase in the near future. These processes can potentially release into the environment a number of substances capable of reacting with biological systems. As fly ash will be a significant coal-derived pollutant, it is important to determine its potential hazard to human health.

F. G. Heuter, Ph.D.
Director
Health Effects Research Laboratory

ABSTRACT

To evaluate the environmental impact of different energy technologies, fly ash samples collected from a coal-fired and from an oil-fired electric power plant were used in aerosol inhalation exposures of mice. The effects of multiple 3-h exposures to the fly ash particles at 2 and 1 mg/m^3 aerosol mass concentration and <0.5 μm MMAD were evaluated in male and female mice by examining the changes in their pulmonary free cells, in their susceptibility to streptococcus infection, and in the bactericidal activity in their lungs to inhaled *K. pneumoniae*. Generally, no consistent differences could be discovered in the effects of the exposures between the two sexes. However, in a combined evaluation of both sexes more and greater significant changes relative to controls were observed in the experimental parameters after inhalation of the oil power plant fly ash than after exposure to the coal fly ash. Thus, the overall results of the study indicate that the pulmonary defense system of mice was more adversely affected by the oil-fired power plant fly ash, a true stack emission effluent, than by the coal fly ash collected by electrostatic precipitator, an in-plant control device.

This report was submitted in fulfillment of EPA Grant No. R805317 by the Life Sciences Division of IIT Research Institute under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period August 1, 1977, to July 31, 1980, and work was completed as of October 31, 1980.

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LIST OF ABBREVIATIONS

ATP	-- adenosine triphosphate
DMSO	-- dimethyl sulfoxide
HEPA	-- high efficiency particulate air filter
MMAD	-- mass median aerodynamic diameter
THB	-- Todd Hewitt broth

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Catherine Aranyi served as principal investigator and James Fenters was co-investigator. The principal professional associate was Jeannie Bradof. Other personnel participating in the program were Michael Biel, Carlos Brown, Richard Cygan, John Hingeveld, Michael McNeal, Carlos Paige, and William O'Shea. The aerosol generation and monitoring system was designed by personnel of the Fine Particles Section of IITRI's Chemistry Division under EPA Contract No. 68-02-2451 (IITRI Project C6380). The radioactive-infectious aerosol chamber was designed in the Life Sciences Division and built with funding provided by IITRI.

SECTION 1

INTRODUCTION

For a correct assessment of the new energy technologies, examination of the impact on health of known or anticipated environmental contaminants resulting from these processes represents a significant part of overall planning. Quantification of health effects resulting from exposure to ambient levels of energy-related particulate effluents will aid in defining, comparing and evaluating conventional and advanced energy-producing processes and thus provide basis for the design of control measures and contribute to the establishment of the air quality and other standards.

Our studies examined under carefully controlled conditions the effects of exposure to aerosols of respirable-size particulate by-products originating from a coal-fired and an oil-fired electric power plant on pulmonary defense mechanism. The overall objectives were to determine if inhalation of these aerosols in short-term and intermediate multiple 3-hr exposures produces changes in number and function of alveolar macrophages or potentiates an experimentally induced infection. Mice were exposed to aerosols of test compounds and the effect of inhalation of these substances on susceptibility to respiratory infection were examined by determining mortality rates and survival times after challenge with airborne pathogenic bacteria. The effect on bacterial clearance after aerosol exposure was evaluated by measurement of changes in the bactericidal activity to inhaled radiolabeled bacteria in the lungs of the animals. In addition, total number, cellular distribution, viability and adenosine triphosphate levels of cells obtained by lavage from the lungs after aerosol exposure were examined.

Results of these studies were summarized in a series of quarterly progress reports (IITRI Report Nos. L6096-1 through 11).

SECTION 2

CONCLUSIONS

The objective of this study was to determine the health effects of potentially fugitive airborne by-products of various energy producing technologies. The <3 μm aerodynamic-size-fraction of a fly ash collected from an in-plant pollution control device (electrostatic precipitator) in a conventional coal-fired electric power plant was compared with the fine particle size fraction of a fly ash collected from the stack emission of an oil-fired power plant. Inhalation exposure to the oil fly ash produced more adverse health effects than exposure to the coal fly ash.

Male and female mice were exposed for 3 hr per day, 5 days weekly, to aerosols of the coal power plant fly ash at 2028 and 1030 $\mu g/m^3$ (0.46 μm MMAD) or to the oil power plant fly ash at 1993 and 1053 $\mu g/m^3$ (0.15 μm MMAD). After 5, 10 or 20 exposures the effects of the fly ash samples on the mice were evaluated by examining if inhalation of the particles had caused changes in the number, composition and function of the free cells lavaged from their lungs, impairment in their pulmonary bactericidal activity to *Klebsiella pneumoniae* and in their susceptibility to experimentally induced streptococcus infection. The results demonstrated no differences in the effects due to sex. However when comparing all experimental parameters and all conditions tested across both sexes, the oil-fired power plant fly ash produced more statistically significant changes than the coal fly ash. This trend was found generally also for each individual experimental parameter with the exception of the ATP levels in pulmonary free cells. Thus there were more significant changes in the total number of cells lavaged from the lungs, in the susceptibility to respiratory infection and in pulmonary bactericidal activity after inhalation of the oil fly ash than the coal fly ash.

We found that similarly to a previously examined copper smelter dust (EPA Grant No. R805141), water-soluble compounds were released from the oil fly ash particles that were cytotoxic to alveolar macrophages *in vitro*. However this was not the case for any of the coal fly ash samples investigated. Since inhalation of both the copper smelter dust and the oil fly ash also significantly depressed pulmonary bactericidal activity and increased susceptibility to respiratory infection, whereas the coal fly ashes did not, we believe that there may be a correlation between the relative inhalation hazard of an aerosol and the partial solubility of its constituent particles in an aqueous millieu, although more research must be performed in this area to prove the hypothesis.

SECTION 3

RECOMMENDATIONS

We recommend the examination of the effects on health of other energy technologies capable of generating by-products that can become potentially hazardous environmental air contaminants. The original plans of this project called for comparison of effluent by-products of conventional and advanced coal conversion technologies. Because of the limited number of advanced process sites available for sampling at the time of commencing these studies, the emission sample from an oil-fired power plant was substituted for examination. A fly ash collected from an in-plant control device was used to represent the by-product of a conventional coal conversion process. The results of our investigations provided important information demonstrating that this oil power plant fly ash represented more of an inhalation hazard than the coal fly ash examined. However the ultimate objective, in view of the abundant coal resources of the country, is comparison and evaluation of the various coal-based energy technologies. If oil-based processes are becoming of interest, the by-products of extracting oil from oil-shale should be considered for their impact on health. In addition future plans should concentrate on collecting true stack emission samples to make a consistent and meaningful evaluation of the potential inhalation hazard contributed to the environment by fugitive emissions of energy technologies possible.

Based on our observations in this and other studies conducted for the EPA on the effects of the solubility characteristics and cytotoxicity of the test particles on their potential inhalation hazard as aerosols, we recommend preliminary testing for solubility in aqueous media and/or *in vitro* prescreening of the particles with the Rabbit Alveolar Macrophage assay.

SECTION 4

EXPERIMENTAL PROCEDURES

Fly Ash Test Samples

Two fly ash samples, one collected from a coal-fired and one from an oil-fired power plant, were supplied by the Inhalation Toxicology Branch, Health Effects Research Laboratory, EPA/RTP. More detailed information on these particulate samples is included in Section 5.

Animals

Male and female CD_1 mice (Charles River Breeding Laboratories, Portage, MI) were used for the inhalation exposure studies. All animals were quarantined for 8-14 days before being used in the studies. During the quarantine period and throughout the experiments, mice were housed in groups of six in stainless steel shoe box cages. Food and water were provided *ad libitum*. During the exposure to the test aerosols and infectious challenge, the mice were housed individually in separate compartments of specially designed stainless steel wire cages.

Inhalation Exposures

All inhalation exposure facilities were located in rooms maintained under negative pressure relative to outside areas. For infectious and radioactive particulates, the aerosol generation and dilution systems as well as the animal exposure chambers were housed in second chamber enclosures (safety cabinet-glove box) that permitted safe handling of the animals and maintenance and monitoring of the experimental environment. The actual inhalation chambers were at all times at negative pressure relative to the outside chamber systems and the latter were negative relative to the pressure in the room. The differential pressures were continuously monitored by Magnahelic pressure gauges (Dwyer, Michigan City, IN). In case of failure in the main exhaust system an alarm (siren) alerted personnel to discontinue aerosol exposures immediately.

Animals were exposed to the experimental environment in various sized (87 to 453 liter) Plexiglas chambers that can hold for 3 hr periods up to 216 mice confined in individual compartments of wire cages. To prevent a build-up of ammonia during the exposure of animals, deotized cage boards (Upjohn Co., Kalamazoo, MI) were placed on the floor of the chambers. Temperature in the exposure chambers was maintained at 24 $\pm$ 2°C at ambient humidity (<60% RH) by providing adequate airflow. Relative humidity was continuously monitored by Airguide Model 605 humidity indicators (Airguide Instrument Co., Chicago, IL) located in each chamber. An electro-hygrometer (Lab-line Instruments, Inc., Melrose Park, IL) was used to calibrate the Airguide indicators.

Exposure to Aerosols of Test Particles --

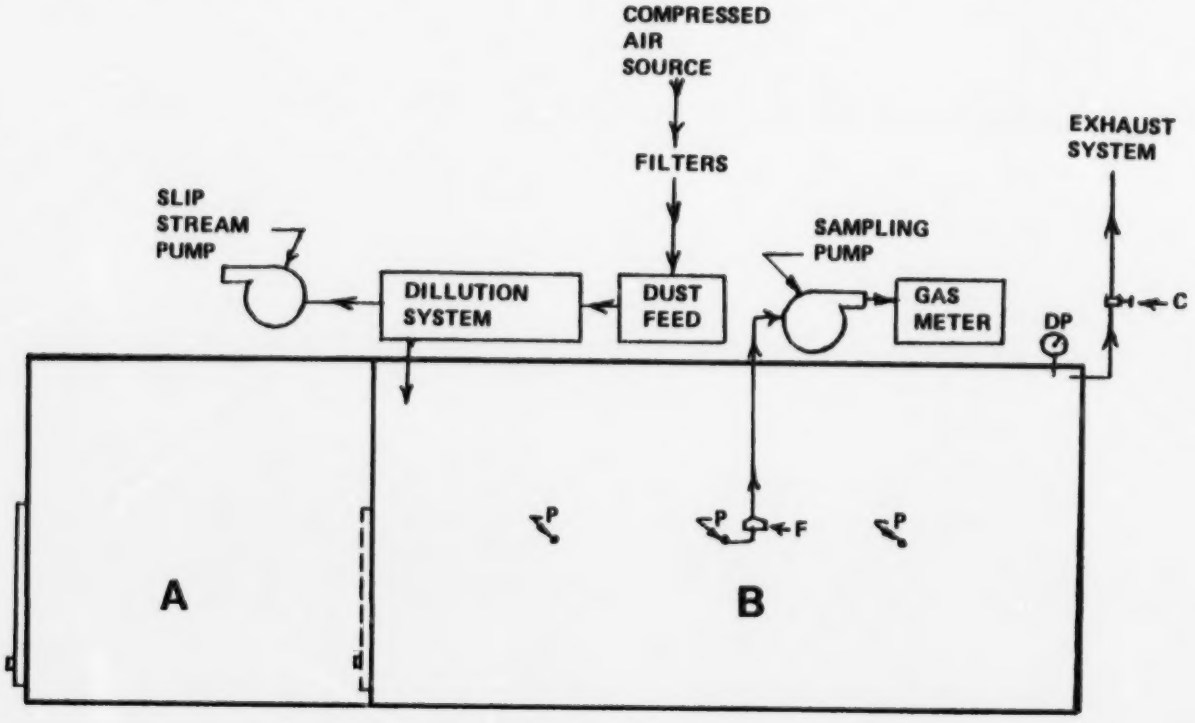
The Plexiglas exposure chamber (Figure 1) consisted of two compartments; a main 453-liter compartment which permitted exposure of 216 mice confined in individual wire cages, and an adjacent 226-liter airlock separated by a sliding door which clamped firmly closed. The various components of the aerosol generation and dilution system i.e., the appropriate filter systems to dry and remove traces of oil and particulates from the compressed air used for dissemination and dilution, along with powder dispersing devices, dilution flasks, a slip stream pump and control gauges were located on a shelf suspended over the exposure chamber.

A teflon-lined pump (Thomas, Industries Inc., Sheboygan, WIS) and a dry gas meter (measuring the air volumes through the Nuclepore sampling filters (Nuclepore Corp., Pleasantown, CA), used for monitoring aerosol mass concentration) were also located on the shelf and could be connected to several sampling ports available at different locations on the chamber.

The aerosol was introduced into the exposure chamber near the bottom at one end and exhausted along the top at the opposite end. Exhaust air flow from the exposure comparment was controlled by a screw-type clamp on the flexible exhaust tubing so that the differential pressure monitored by a Magnahelic gauge could be balanced as required: i.e. the pressure in the exposure compartment maintained negative at all times relative to the room air.

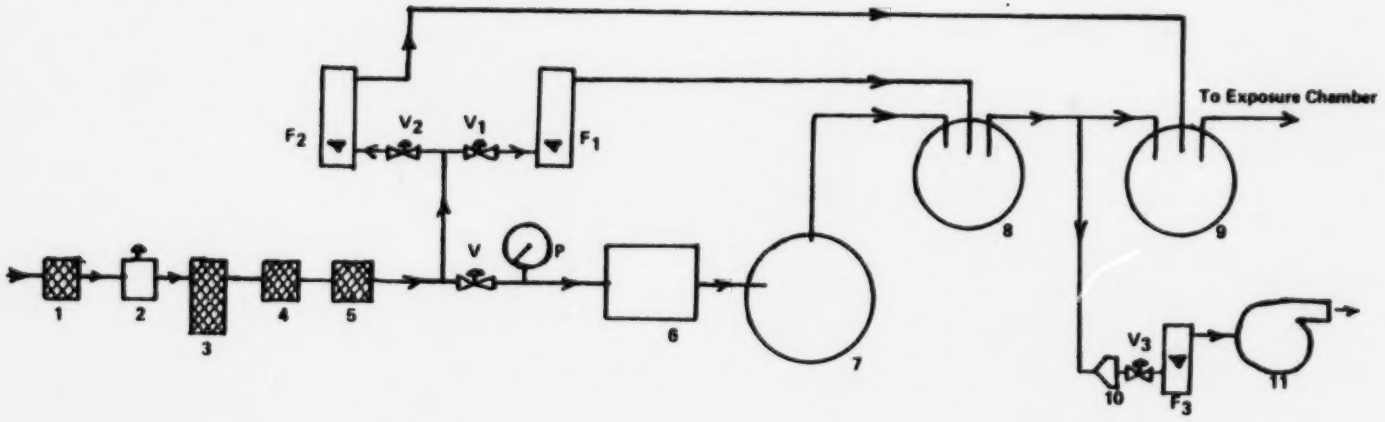
Generation and Monitoring of Aerosols--The schematic diagram of the aerosol generation system used in the studies is shown in Figure 2. The compressed air supplied to the exposure chamber for dilution or dissemination of the test particles was passed through appropriate filter systems to dry the air and to remove all traces of oil and particulates. These included an Alemite coarse filter (Model 7620, Steward Warner, Chicago, IL) to dry the air, followed by a pressure regulator and a Deltech Model 130 oil filter (Deltech Engineering Inc., New Castle, DE). Finally, before entering the chamber the air was further purified by passage through a MSA (Mine Safety Appliance Co., Pittsburgh, PA) absolute filter assembly to remove particles larger than 0.3 μm and a constant flow compressed air filter (Koby Inc., Marlboro, MA). A Wright dust feeder (L. Adams Ltd., London, England) was used to generate aerosols from the two fly ash samples. The material to be aerosolized was compressed into a 13 mm diameter cylinder of the dust feeder. A slowly rotating blade continuously scraped off a small portion of the particles which were then entrained into a 21 liter/min air stream. The dust-laden air was impacted on a plate to break up the agglomerates and then passed through a settling chamber where the large particles were removed by gravity impaction. After appropriate dilution the aerosol was introduced into the exposure chamber

The purified air flow through the dust feeder was controlled by a valve and monitored by a pressure gauge. To obtain the desired mass concentration, the aerosol was passed through a two-stage dilution system consisting of two sequential dilution flasks controlled by two valves and monitored by two flowmeters. The primary system was capable of a three-fold dilution; for lower



A	Airlock for entry of animals
B	Main exposure compartment
C	Clamp controlling exhaust air flow
DP	Differential pressure guage between room and compartment B
F	Collecting filter for gravimetric sampling
P	Sampling ports

Figure 1. Schematic diagram of inhalation exposure chamber to fly ash aerosols



1. Coarse filter
2. Pressure regulator
3. Deltech model 130 oil filter
4. MSA air line absolute filter
5. Koby air purifier and flow equalizer
6. Wright dust feeder
7. Settling chamber
8. Dilution flask #1
9. Dilution flask #2
10. Glass fiber filter holder
11. Vacuum/pressure pump

Valves controlling airflow:

V to dust feeder
V_1 to dilution flask #1
V_2 to dilution flask #2
V_3 through slip stream

Flowmeters for airflow:

F_1 to dilution flask #1
F_2 to dilution flask #2
F_3 through slip stream

P dust feeder pressure gauge

Figure 2. Schematic diagram of solid particulate aerosol generation system.

concentrations a secondary dilution was also used. In addition, any portion of the diluted aerosol could be removed by a vacuum/pressure-pump via a slip stream monitored by a flowmeter. Adequate airflow into the exposure chamber prevented drastic changes in temperature and humidity during aerosol exposures of animals in the chamber. The aerosol was introduced into the exposure chamber near the bottom at one end and exhausted along the top at the opposite end.

Aerosol mass concentration in the chamber was monitored continuously during the exposure using a Model JM-7000 Aerosol, smoke and dust photometer (Phoenix Precision Instruments, Gardiner, NY) and a condensation nuclei counter (Type CN Small Particle Detector, Gardner Assoc., Inc., Schenectady, NY). These two instruments were used to assure that the aerosol concentration was in the required range during the entire exposure period, or if adjustments in the aerosol generation system were needed. Actual mass concentration was obtained in $\mu g/m^3$ from gravimetrically determined filter-collected particles and the corresponding volumes of air measured during the sampling period. (Three filter samples were taken during a 3-hr exposure period.)

The photometer operates on the principle of forward light-scattering, detecting the intensity of light scattered by the suspended particulates sampled. This light intensity is proportional to the mass concentration of the aerosol. The photometer reading also depends on particle size, shape, refractive index and size heterodispersity and therefore the instrument has to be calibrated for the particular light-scattering qualities of each experimental powder. Readings on the condensation nuclei counter depend on the number of particles in the sampled aerosol. Both instruments are calibrated by gravimetric analysis of samples collected on membrane filters.

For the gravimetric determination, samples of aerosols diluted to various mass concentrations were collected during the exposure period on 0.1 μm Nuclepore filters at constant flow rates using an oilless, teflon-lined pump (Model 907CA18-TFE, Thomas Industries, Inc., Sheboygan, WI). A dry gas meter (Model F-190 Rockwell International, MCU Div., Pittsburgh, PA) connected to the back side of the pump recorded the total volume of air sampled. Samples collected for sufficient time intervals such that the weight of particles collected was 5 to 10% of the weight of the membrane filter were weighed on a Mettler M30 electronic microbalance (Mettler Instrument Corp., Hightown, NJ) in the presence of a static eliminator. Air temperatures in the exposure chamber and at the dry gas meter were recorded so that the volume of air sampled and totalled by the gas meter could be corrected to chamber temperature at which the gravimetric sample was collected.

Aerosol particle size distribution was monitored with a piezoelectric microbalance particle cascade impactor (California Measurements, Sierra Madre, CA). This instrument combines the reliability of cascade impaction with the sensitivity of quartz crystal microbalances. Unlike conventional cascade impactors in which particulates are first collected over periods of hours and then weighed on a separate external balance, the particulate mass in the piezoelectric particle microbalance is weighed *in situ* and in real-time on the impaction surface where it is collected. Thus, the aerosol mass and size information is obtained directly during the sampling.

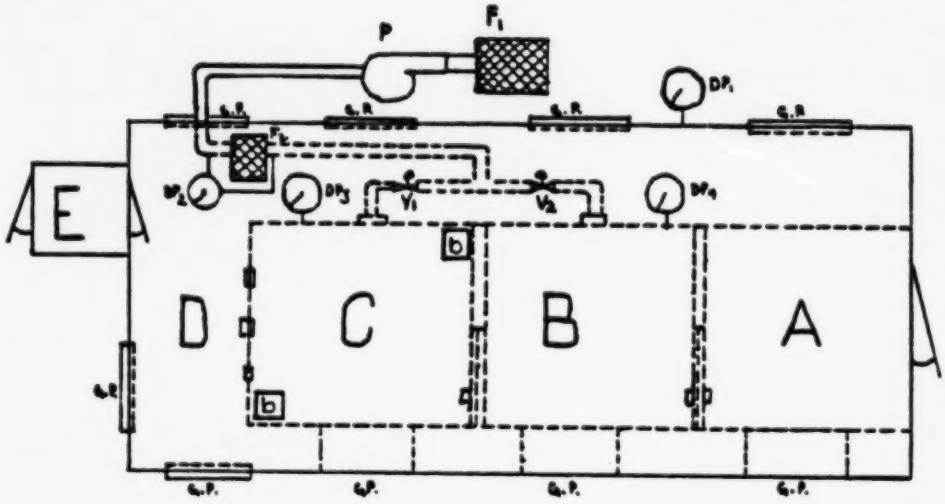
Exposure to Bacterial Aerosols--

Infectious aerosol exposure--Mice were challenged with bacterial aerosol in a 380-liter Plexiglas chamber installed within a microbiological safety cabinet. In this chamber, 144 mice housed in individual compartments of stainless steel wire cages can be exposed to the aerosol simultaneously. Temperature and humidity in the aerosol chamber were maintained at 24 + 2°C and 65 ± 5% RH. A continuous flow nebulizer (DeVilbiss Model 841, The DeVilbiss Co., Somerset, PA) was used to produce the bacterial aerosols with particles of <4 μm mass median diameter (MMD) as determined by a cascade impactor. Filtered air was supplied to the inlet of the nebulizer at a flow rate of 8 liters/min. Secondary air supply was maintained at 28 liters/min. The aerosol was sampled with an AGI-30 glass impinger (Ace Glass Inc., Vineland, NJ) containing 0.1% peptone as a collecting fluid. Upon completion of each bacterial aerosol exposure, ultra-violet germicidal lamps were used inside the safety cabinet and inside the exposure chamber, the exhaust air flow was increased, and the exposure chamber was purged with clean air.

Streptococcus pyogenes (Lancefield Group C) was used as the infectious challenge agent. To maintain the stock culture, mice were injected intraperitoneally with a suspension of *Streptococcus* and killed 24 hr later. Heart blood was incubated on blood agar for 48 hr at 37°C and *Streptococcus* colonies isolated from the agar were inoculated on blood agar with Todd Hewitt broth (THB) as the base. After 24-48 hr at 37°C, bacteria were harvested into Difco Nutrient broth (Difco Laboratories, Detroit, MI) with added 15% glycerol (v/v) and 1 ml aliquots were frozen at -70°C. For dissemination, 0.8 ml of the thawed bacteria was grown in THB at 37°C for 18 hr, harvested by centrifuging at 10,000 xg for 10 minutes, resuspended in 0.1% peptone water, and the concentration of bacteria adjusted to 10% transmittance in a Spectronic 20 spectrophotometer (Bausch & Lomb Inc., Northbrook, IL) at 440 mμ.

The inhaled dose of bacteria was determined by killing one mouse from each exposure group immediately after the infectious challenge. The lungs were aseptically removed, weighed, and homogenized in 2.0 ml of sterile 0.1% peptone water. The suspension was diluted and plated onto blood agar and the colonies were counted after 48 hr incubation at 37°C. The inhaled dose expressed as number of viable bacteria per lung generally ranged from 10 to 30 x 10^3 organisms.

Radioactive bacterial aerosol exposure--The chamber was installed in a glove box (Figure 3). To further prevent radioactive and pathogenic exposure hazard to laboratory personnel all air exhausted from the chamber system was passed first through an absolute filter (Model M. Watts Regulator Co., Lawrence, MA) placed within the glove box and subsequently through a HEPA filter located on the outside. The 87-liter main compartment of the chamber was accessible through appropriate airlocks to introduce or remove the animals, and permitted exposure of up to 72 mice held in individual compartments of wire cages. At the opposite end of the safety cabinet from the door through which the animal cages were passed, an additional small airlock provided an entry port for the nebulizer and impingers, as well as for the exchange of the exhaust line filters. Two biscuit blowers (Rotron Inc., Woodstock, NY),



A,B	Airlocks for entry of animals
C	Exposure chamber
D	Safety cabinet
E	Airlock for entry of miscellaneous equipment
G.P.	Glove port
F_1	HEPA filter
F_2	Absolute filter
P	Blower (air pump)
b	Biscuit blowers
DP	Differential pressure gauges:
	DP_1 between room and safety cabinet, D
	DP_2 across absolute filter
	DP_3 between compartments C and D
	DP_4 between compartments B and D
V	Valves controlling:
	V_1 exhaust airflow from compartment C
	V_2 exhaust airflow from compartment B

Figure 3. Schematic diagram of radioactive bacterial aerosol exposure chamber.

controlled by a variac, assured homogeneous dispersion of the aerosol in the exposure chamber. The aerosol was produced by a Retec X-70 disposable nebulizer (Cavitron KDC, Inc., Anaheim, CA) using compressed air passed through appropriate filter systems. Airflow to the nebulizer was controlled by a flowmeter and passed through an electric solenoid valve, whereby aerosolization could be turned off using a timer. Secondary dilution air to the system provided adequate air-flow to maintain uniform temperature and humidity during the aerosol exposure (Figure 4).

Before aerosolization total bacterial counts (BA) in a Petroff-Hausser microscopic counting chamber (Hausser Scientific, Blue Bell, PA) and radioactive counts (RA) by a liquid scintillation counter (Searle Analytic Inc., DesPlaines, Il) were determined on the bacterial suspension to establish the concentration and radioactive labeling ratio (K = BA/RA). The radioactive labeling of the bacteria was adequate for monitoring if K <50. Based on the microscopic counts, the nebulizer concentration was adjusted to 6×10^9 bacteria per ml. To obtain the required bacterial deposition in the lungs from the aerosol, approximately 15 ml of this bacterial suspension were aerosolized during a 30 min period with air flow rates of 6.5 liter/min through the nebulizer and 13 liter/min for secondary dilution air.

Cultures of ^{35}S-labeled *Klebsiella pneumoniae* were grown in Anderson's medium. The magnesium sulfate in the medium was replaced by magnesium chloride and the sulfate requirement of the bacteria was provided by addition of ^{35}S-labeled sodium sufate in a specific activity range of 600-1000 μCi/mM (New England Nuclear, Boston, MA). After harvest, the bacteria were washed and centrifuged at 12,000 xg 10 times for removal of unattached radiolabel. All bacterial counts were initially determined by dark field microscopy in a Petroff-Hausser bacterial counting chamber and subsequently also by culture plate technique.

Radioactive counts were measured with a Mark III Liquid Scintillation System Model 6880 (Searle Analytic Inc., Des Plaines, IL) using samples channels ratio to compute counting efficiency. The ^{35}S isotope adsorbs to the glass vials when liquid scintillation cocktails are used, resulting in up to 30% losses in total radioactive counts of the samples. To prevent this adsorption and to better suspend the lung homogenates, the samples for scintillation counting were prepared as gels, formed by adding 6.0 ml of Aquasol-2 (New England Nuclear, Boston, MA) and 3.0 ml of water to 1.0 ml aliquots of the lung homogenates or the bacterial suspensions.

Measurement of Experimental Parameters

Effects of 5, 10 or 20 daily 3-hr exposures to aerosols of the test particulates on mice were evaluated by observation of changes in their susceptibility to respiratory bacterial infection, pulmonary bactericical activity to inhaled radiolabeled *K. pneumoniae*, and by examination of the pulmonary free cells lavaged from their lungs. The effects were assayed within 1 hour after the last exposure to aerosols of the test particulates.

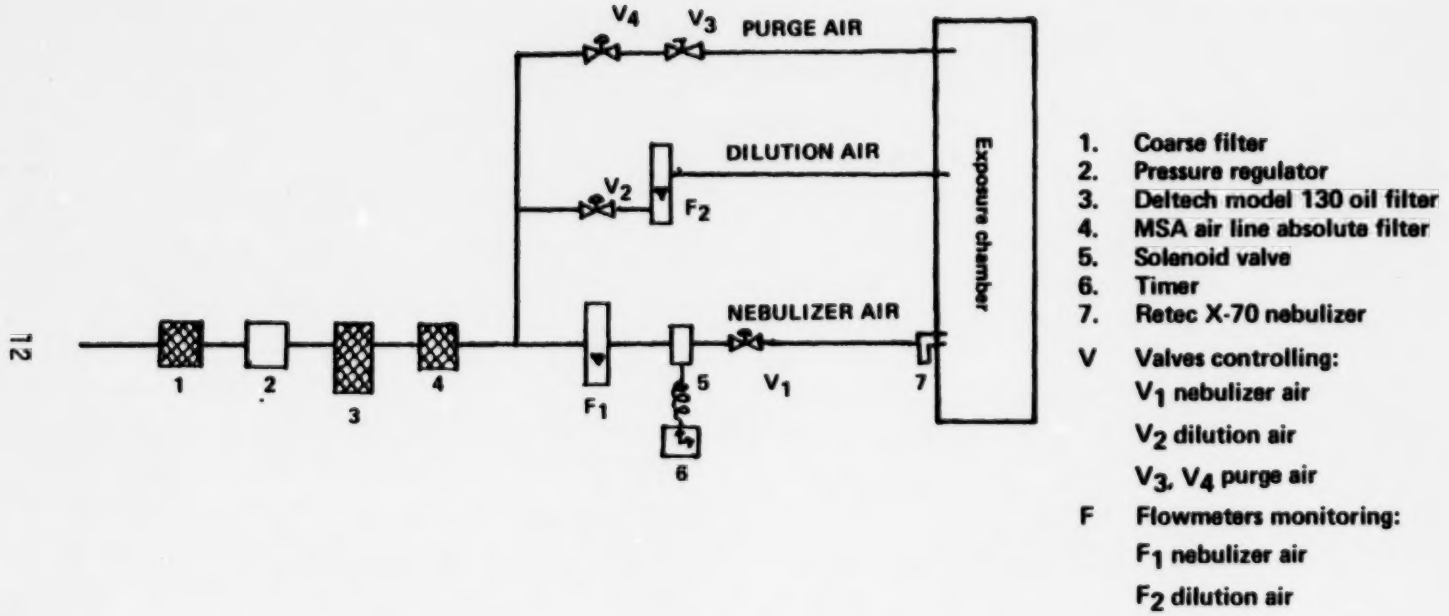


Figure 4. Schematic diagram of radioactive bacterial aerosol generation system.

Infectivity--

In the experiments using the infectivity model system, animals exposed to the hazardous air pollutants and those exposed to filtered air were challenged simultaneously with the infectious agent so that mortality rates and survival time could be determined and compared within a given experiment. After the challenge, the mice were removed from the chamber and held in a clean-air, isolated animal room. During the 14-day observation period, deaths were recorded daily. The mean survival time was estimated according to the following equation:

$$MST = \frac{\Sigma(AxB)+(dxL)}{n}$$

where, A is the last day on which any individual mouse is alive, B is the number of mice surviving A days, d is the last day of observation, L is the number of mice alive on day d, and n is the number in the experimental group.

Pulmonary Bactericidal Activity--

Intrapulmonary bacterial inactivation was determined in the lungs of individual animals with a modification of the method of Green and Goldstein (13), whereby mice were exposed to radiolabeled live bacteria. The ratio of the viable bacterial count to the radioactive count in each animal's lung provided the rate at which bacteria were destroyed by the lung in a given time after infection. After exposure to the test compounds, groups of mice were infected by exposure to aerosols of ^{35}S-labeled *K. pneumoniae*. Immediately (0 hr) and at 3 hr after the infectious challenge, 5 (0 hr) and 12 (3 hr) mice, respectively, from each treatment group were killed and their lungs were aseptically removed and homogenized. From the radioactive counts made on aliquots of the homogenate and the radioactive labeling ratio determined for each bacterial culture in 0-hr lung samples, the total number of bacteria deposited in each lung was calculated. On the basis of this information another aliquot of each lung homogenate was appropriately diluted to determine by plate count technique the viable bacteria that remained in the lungs.

Pulmonary Free Cells Lavaged from the lungs--

Mice were killed by intraperitoneal injection of sodium pentobarbital and the lung-heart-trachea complex removed from the chest cavity *in toto*. A blunted hypodermic needle inserted into the trachea and fastened with surgical suture was used to lavage the lungs by repeated infusions of warm saline.

Isolation and characterization of cells--The cells were isolated from the lavage fluids by centrifuging at 365 xg and washing in Hanks' medium. Total cell counts were made using a hemocytometer. To determine the percent distribution of alveolar macrophages, polymorphonuclear leukocytes and lymphocytes, differential counts were made on smears of cells obtained by cytocentrifuge, fixed in methanol and stained with Wright's stain. Viability was determined by dye exclusion using 1% trypan blue. At least 200 cells selected at random were counted microscopically to determine differential counts and viability.

Measurement of cellular ATP--Cellular adenosine triphosphate (ATP) concentrations in the lavaged cells was determined using a DuPont 760 Luminescence Biometer with the procedure recommended for the instrument. The assay is based on the principle that when a microsample containing ATP is injected into a suitably buffered reaction mixture of luciferase and luciferin, the peak intensity of the resulting light flash is directly proportional to the concentration of ATP. ATP was extracted from the cells by dimethyl sulfoxide (DMSO) from aliquots of the lavaged and washed cellular suspension. The extracts were diluted with 0.01 M morpholinopropane sulfonic acid buffer to overcome the quench effect of the high concentration of DMSO in the aqueous extract for the luciferase-luciferin reaction. ATP levels were expressed as femtograms (10^{-15}g) of ATP per 10^5 cells (calculated from initial cell counts).

Data Anaylsis

Results of the experiments were subjected to statistical analyses and the significance of the observed differences were reported at the 10% probability level. As appropriate, Chi-square test, Student's t test, and two-way analysis of variance were used.

SECTION 5

RESULTS AND DISCUSSION

The coal-fired power plant fly ash provided by EPA originated from high-sulphur-containing Eastern coal and was collected as an electrostatic precipitator hopper ash from a conventional power plant. Since this fly ash was not collected in samplers located after the in-plant pollution control devices, it cannot be considered as a true effluent sample and is not necessarily similar in chemical composition to true emission samples. However, study of the physical characteristics and elemental composition of this fly ash by Griest and Guerin (ref. 1) demonstrated that the <3 μm aerodynamic size fraction used in the present studies can act as a qualitative surrogate for the constituents adsorbed on the stack ash. The most prevalent trace metals according to that study were As, Ba, Co, Cr, Cu, Pb, Sr and Zn; the presence of polycyclic aromatic hydrocarbon compounds was also established.

In vitro examination of this coal fly ash sample by the Rabbit Alveolar Macrophage (RAM) test was conducted in our laboratory as part of another study supported by EPA (Grant No. R805141). Using viability, total cellular protein content and ATP levels as experimental parameters we found (ref. 2) this fly ash to be much less cytotoxic than copper or aluminum smelter dusts, but more cytotoxic than fly ash samples from two other conventional- and one fluidized-bed-coal-conversion processes, or particles from two steel manufacturing plants. These studies also demonstrated that under the given procedures, no soluble components cytotoxic to alveolar macrophages were released from the fly ash particles into the incubation medium. (In these recently completed studies this fly ash sample was referred to as Coal Fly Ash No. 4).

The oil-fired power plant fly ash was collected from stack emission at 400°F in a fabric filter collection jar as a true effluent sample, not from an in-plant control device. We were provided a fine particle size fraction. Sulphur and vanadium are among the known inorganic constituents of the crude oil. No other analytical information has been made available to us by the EPA yet.

During collection it was observed (P. Cavanaugh, Southern Research Institute; personal communication) that due to absorbing moisture from the atmosphere the material changed color and consistency from a light grey powder to a non-dispersable hard green crust when maintained under ambient conditions in an open container, but did not change during storage in a closed jar. Throughout the several months of our aerosol exposure studies, we never observed such changes in the powder accumulating in the settling and dilution flasks of the aerosol generation system, nor did we have any difficulty in dispersing the fly ash with a Wright Dust Feeder when we used filtered, dried air for dissemination and dilution of the aerosols. However after the studies were completed and the exposure chamber system was opened to ambient air, the transformation described above indeed took place.

Exploratory *in vitro* testing in our laboratory of this fly ash with the RAM assay appeared to indicate a greater relative cytotoxicity than what we have documented for the coal fly ash. In addition we could demonstrate that soluble components (vanadium was analytically established) cytotoxic to alveolar macrophages were released during *in vitro* incubation from this oil power plant fly ash (unpublished data).

Male and female mice inhaled aerosols of the coal-fired power plant fly ash or the oil-fired power plant fly ash at 1 and 2 mg/m^3 mass concentration in multiple daily (5 days/week) 3-hr exposures. The aerosol mass concentrations were determined by taking the averages of three daily filter readings and calculating the means and standard deviations (SD) over the total number of exposure days. The means and SD for the coal fly ash were 2028±322 µg/m^3 and 1030±187 µg/m^3; and for the oil power plant fly ash 1993±222 µg/m^3 and 1053±208 µg/m^3. The aerosols had log-normal distribution with mass median aerodynamic diameters and σ_g-s of 0.46 µm and 2.1 for the coal power plant fly ash and 0.15 µm and 3.1 for the oil power plant fly ash, respectively.

The effects were determined after 5, 10 and 20 exposures by examining changes in the free cells lavaged from the lungs, susceptibility to respiratory streptococcal infection, and pulmonary bactericidal activity to inhaled radiolabeled *K. pneumoniae*. Results are summarized in Table 1 and are expressed for the pulmonary free cell data as percentage of the control responses, and for mortality rates (%), mean survival times (days) and pulmonary bactericidal activity (%) as the change between exposed and control observations.

Changes in the total number and ATP content (representing phagocytic competence) of free cells lavaged from the lungs were clearly affected by inhalation of the two fly ash samples (Figure 5). Total cell counts were frequently quite elevated in exposed animals relative to controls. Aerosols of the coal fly ash at 2028 µg/m^3 produced significant increases after 5 and 10 exposures in male and after 10 and 20 exposures in female mice. After exposure to 1030 µg/m^3 of the coal fly ash there were no significant changes in total cell counts in either sex with the only exception of 10 exposures in male mice.

Following inhalation of the oil power plant fly ash significant increases in total cell counts occurred in both male and female mice after 10 and 20 exposures at 1993 µg/m^3 as well as at 1053 µg/m^3 aerosol mass concentration. Differential counts and percent viability of the lavaged cells (not shown in the table or figure) were not affected by exposure to either of the fly ash samples under any of the experimental conditions used.

Cellular ATP levels were generally depressed following inhalation of the coal power plant fly ash. Although exposure to 2028 µg/m^3 of the coal fly ash did not produce significant changes in male mice, significant decreases were seen after 5, 10 and 20 exposures to 1030 µg/m^3 of the aerosol. In female mice 5 and 10 exposures to 2028 µg/m^3 or to 1030 µg/m^3 of the coal fly ash produced significant decreases.

The changes in ATP levels of pulmonary free cells after exposure of mice to aerosols of the oil power plant fly ash were fewer in number and more variable. At 1993 µg/m^3 no changes were found in ATP of male mice, whereas a significant decrease was seen after 5 exposures and a significant increase after 20 exposures in female mice. In contrast significantly increased ATP levels were found after 5 and 20 exposures to 1053 µg/m^3 of the oil power plant fly ash in male, but no changes were detected in ATP of female mice at this aerosol concentration.

TABLE 1. EFFECTS OF MULTIPLE DAILY 3-HR EXPOSURES TO AEROSOLS OF A COAL-FIRED POWER PLANT FLY ASH OR AN OIL-FIRED POWER PLANT FLY ASH ON THE PULMONARY FREE CELLS, ON SUSCEPTIBILITY TO RESPIRATORY STREPTOCOCCUS INFECTION AND ON BACTERICIDAL ACTIVITY TO INHALED ^{35}S-*K. PNEUMONIAE* IN CD_1 MICE

Aerosol Exposure					% of Control				% Change: Exposed-Control				
					Total Cells		Cellular ATP		Streptococcus Inf.			Bactericidal Activity	
Sample	Conc, $\mu g/m^3$ Mean	SD	No. of Exposures, 3 hr/day	Sex	n[a]	Count x 10^5	n[a]	10^8fg/10^5 cells	n[a]	Mortality %	MST days	n[a]	% *K. pneumoniae* killed
Coal-Fired Power Plant Fly Ash	2028	322	5	M	84	113**	76	94	382	0	0	47	6.6**
			10		18	205**	16	91	395	-0.1	0.1	45	-2.0
			20		49	117	49	97	237	8.4	-0.9	23	2.1
	1030	187	5		33	124	32	85**	318	3.7	-0.4	45	2.7*
			10		32	150**	32	78**	267	-2.1	0.4	40	0.4
			20		32	94	32	73**	296	4.9*	-0.4*	36	-3.1
	2028	322	5	F	67	96	56	89**	475	5.9**	-0.2	58	3.6*
			10		23	140*	16	60***	385	5.3	-0.6	44	1.7
			20		19	147*	15	113	137	17.9**	-1.2**	24	1.4
	1030	187	5		32	109	30	85**	295	1.1	-0.1	43	-2.3
			10		51	111	47	82**	375	0.1	0.1	86	-0.9
			20		74	105	68	99	334	4.5**	-0.6*	61	6.7***
Oil-Fired Power Plant Fly Ash	1993	222	5	M	31	107	31	97	278	18.2***	-1.6***	43	2.7*
			10		32	224***	32	106	229	18.8***	-1.5***	38	-4.0**
			20		32	165***	32	94	306	10.7**	-0.9***	40	3.8
	1053	208	5		36	95	33	116*	237	-0.7	-0.1	44	1.1
			10		34	134**	32	102	145	9.3**	-0.8**	45	-2.4
			20		32	140**	31	128**	273	-5.6**	0.4**	44	0.8
Oil-Fired Power Plant Fly Ash	1993	222	5	F	32	104	31	85**	330	25.2***	-2.5***	43	-5.4**
			10		35	178***	31	96	310	5.1*	-0.5*	44	-2.1
			20		33	178***	31	118**	146	2.6	-0.4	44	1.7
	1053	208	5		34	115	33	112	297	1.9	-0.2	46	-2.5*
			10		37	153***	30	89	239	-0.1	0	43	1.5
			20		34	177***	33	96	212	-12.3**	0.7**	45	-3.5*

[a] Number of mice

Significant change from corresponding control mice determined by two-way analysis of variance (total cell counts, ATP/cells); Chi-square (% mortality) and Student's t-test (Mean Survival Time; % bactericidal activity): *$p \leq 0.1$ **$p \leq 0.05$ ***$p \leq 0.001$.

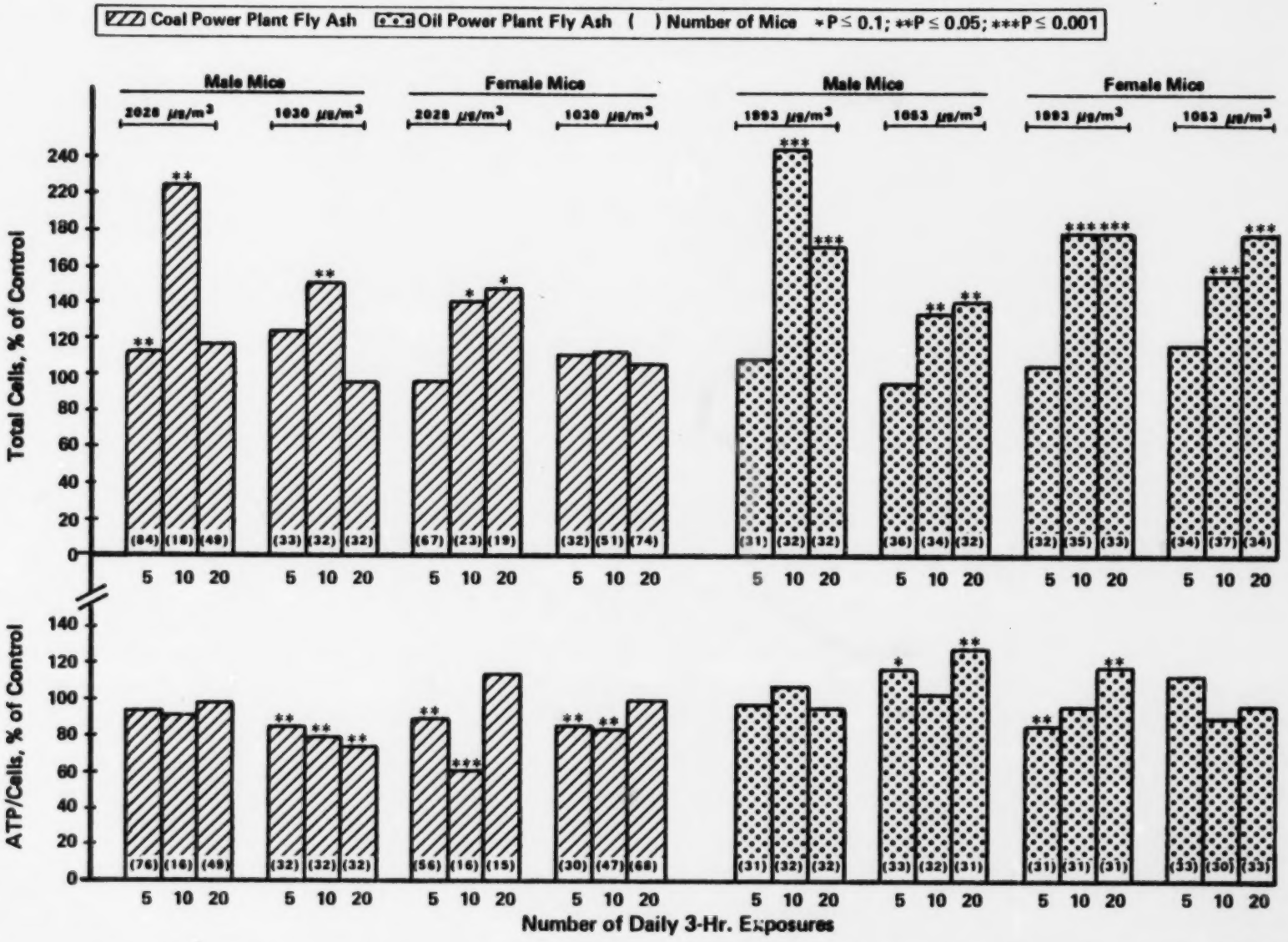


Figure 5. Effects of multiple 3-hr daily exposures to aerosols of Coal Power Plant Fly Ash or Oil Power Plant Fly Ash on total cell counts and cellular ATP levels determined in the pulmonary cellular lavage of mice.

The significant increases found in total cell counts after exposures to the oil power plant fly ash could have contributed to the inconsistency of the ATP levels if the influx of new cells had different and/or more variable ATP contents from the resident macrophages. However, since according to the differential counts, the cellular distribution did not change with the increasing cell numbers, it can only be speculated that different degrees of maturity of the new macrophages could be responsible for the variability in ATP levels.

In susceptibility to inhaled streptococcal infection, a greater number of significant changes was observed following exposure to the oil-fired power plant fly ash than after inhalation of the coal fly ash aerosols. Figure 6 shows the difference in percentage mortality due to streptococcal pneumonia between aerosol-exposed and filtered air-exposed control mice. Exposure to aerosols of the coal fly ash generally did not significantly affect mortality in male mice except for an increase following 20 daily 3-hr exposures at 1030 $\mu g/m^3$. In female mice mortality rates were significantly increased after 5 and 20 exposures to 2028 $\mu g/m^3$ and following 20 exposures to 1030 $\mu g/m^3$ of the coal fly ash aerosol.

In contrast, inhalation of the oil power plant fly ash produced more significant changes in susceptibility to streptococcal infection in male than in female mice. At 1993 $\mu g/m^3$ aerosol mass concentration mortality was significantly increased in male mice after 5, 10 and 20 daily 3-hr exposures and after 5 and 10 exposures only in female mice. However, there were fewer mice in the female 20-exposure group and the absence of a statistical significance may be a result of the insufficient numbers. At 1053 $\mu g/m^3$ there was no change in mortality of male mice after 5 and a significant increase after 10 exposures to the oil power plant fly ash; however following 20 daily 3-hr exposures significantly decreased mortalities from streptococcal infection were observed in both male and female mice. Five and 10 exposures at this concentration did not produce significant mortality changes in female mice.

Mean survival times (Table 1) showed significant decreases paralleling the significant increases in mortality rates for both of the fly ashes except after 5 exposures to 2028 $\mu g/m^3$ of the coal fly ash aerosol in female mice. Significant increases in mean survival time occurred correlating with the significant mortality decreases in both sexes after 20 exposures to aerosols of the oil power plant fly ash at 1053 $\mu g/m^3$.

The bactericidal activity to inhaled radiolabeled *K. pneumoniae* (Figure 7) was significantly altered in relatively few exposure combinations with either of the fly ash samples. Following exposure to aerosols of the coal fly ash all significant changes were increases compared to controls. At 2028 $\mu g/m^3$ aerosol mass concentration 5 daily 3-hr exposures produced significant changes in both sexes, whereas inhalation of 1030 $\mu g/m^3$ resulted in significant increases after 5 exposures in male and 20 exposures in female mice.

Significant bactericidal activity changes after exposure to the oil power plant fly ash were generally decreases relative to controls with the one exception of 5 daily 3-hr exposures to 1993 $\mu g/m^3$ in male mice resulting in a significant increase. Ten exposures of male and 5 exposures of female mice to the same aerosol concentration produced significant decreases in bactericidal activity. Following 5 or 20 daily 3-hr exposures to 1053 $\mu g/m^3$ of the oil

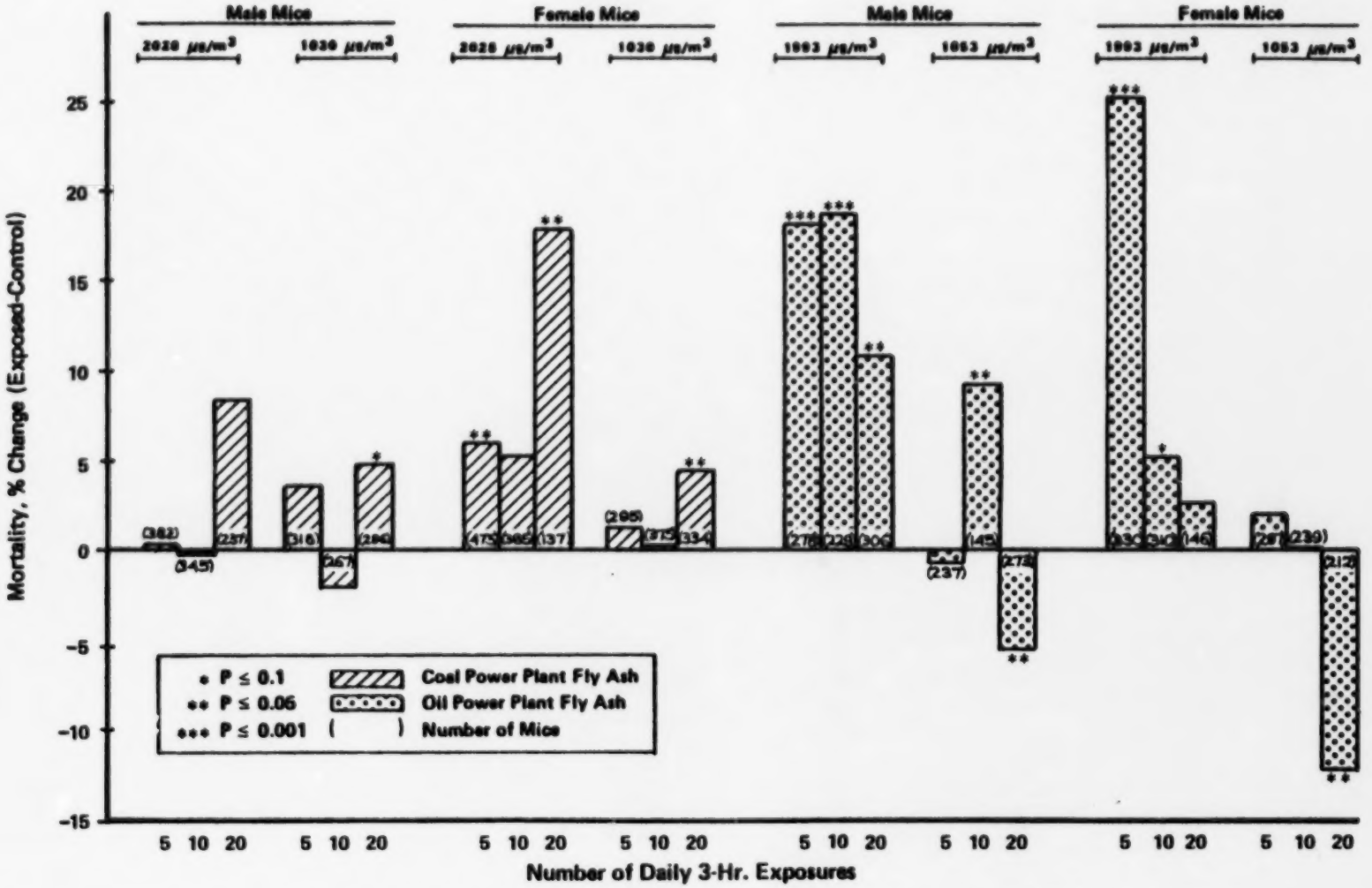


Figure 6. Changes in mortality in mice from streptococcal pneumonia after multiple 3-hr daily exposures to aerosols of Coal Power Plant Fly Ash or Oil Power Plant Fly Ash.

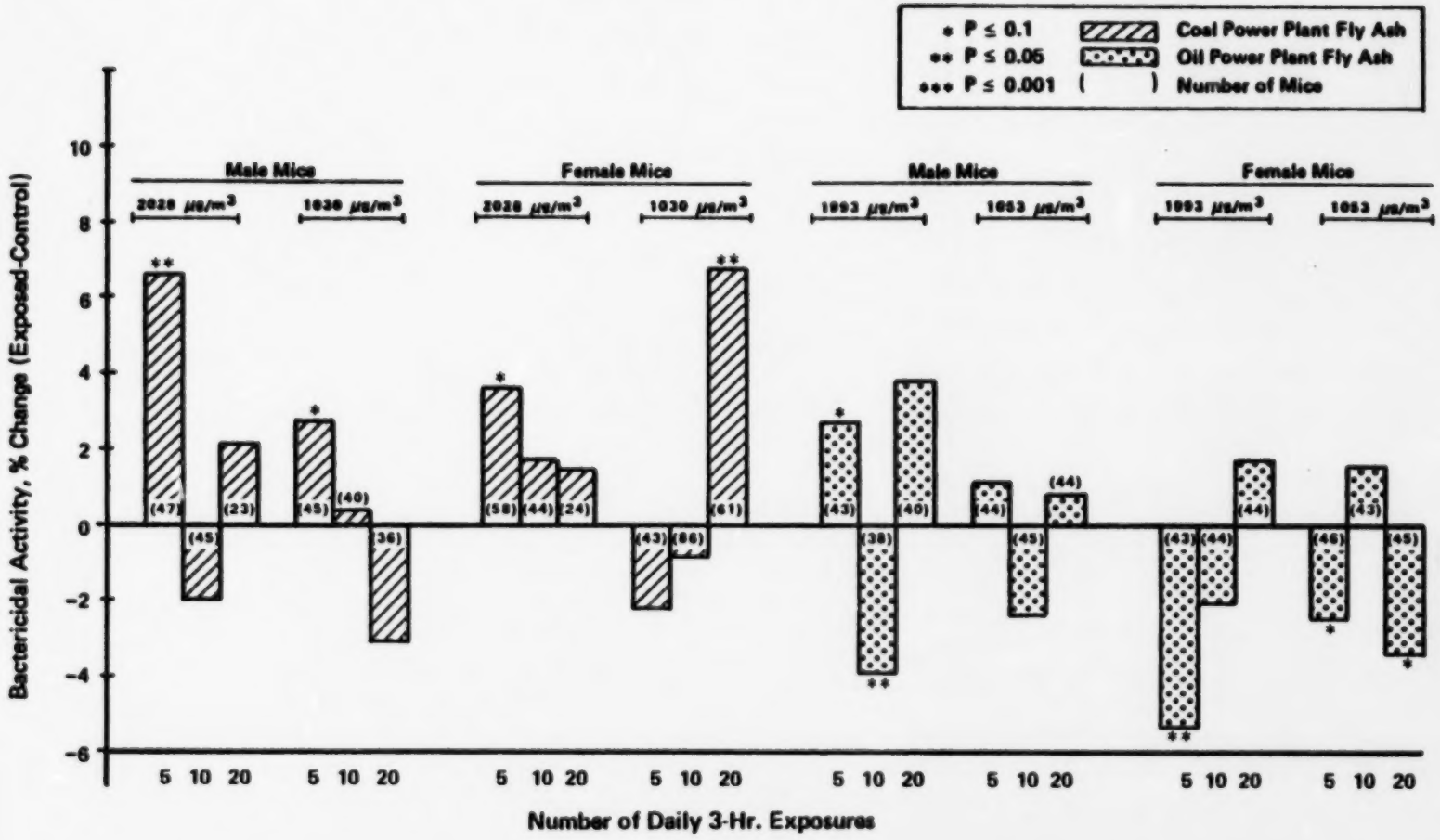


Figure 7. Changes in pulmonary bactericidal activity in mice to inhaled ^{35}S-*K. pneumoniae* after multiple 3-hr daily exposures to aerosols of Coal Power Plant Fly Ash or Oil Power Plant Fly Ash.

power plant fly ash pulmonary bactericidal activity was significantly decreased in female mice but no significant changes were observed in male mice after any of the multiple exposures at this aerosol mass concentration.

Thus, in general, the changes in bactericidal activity following inhalation of the coal fly ash were increases compared to the control values. Changes after exposure to aerosols of the oil power plant fly ash were decreases, typical of what we have observed in the past with more toxic particles (ref. 2).

For a better review and comparison of the effects of the two fly ash samples the overall results in terms of significant increase, decrease or no change are presented in a summary table (Table 2). It is evident from the table that comparison by sex only shows generally almost equal numbers of significant changes in male and female mice *in toto* (22 and 23), as well as for each parameter separately (i.e. for males and females respectively the distributions were: total cell count 7 and 6, cellular ATP 5 and 6, mortality 6 and 6 and bactericidal activity 4 and 5). The effects of each individual fly ash sample on the two sexes appeared to be generally similar, with the exception of susceptibility to respiratory infection, i.e., the coal fly ash produced more significant changes in female and the oil power plant fly ash in male mice. In other words, for male and female mice *versus* exposure to the coal- and to the oil power plant fly ash, respectively, the distribution of significant changes in the various experimental parameters was as follows: total cell count 3 and 2 *versus* 4 and 4; ATP 3 and 4 *versus* 2 and 2, bactericidal activity 2 and 2 *versus* 2 and 3, but mortality 1 and 3 *versus* 5 and 3. Thus the results of this essentially qualitative evaluation indicate no more than random variations between the responses in the two sexes. Because experimental logistics prevented it, studies with male and female mice were not conducted simultaneously and the sample sizes were not necessarily always similar. Therefore no in-depth statistical analysis was applied to this aspect of the studies.

In terms of an overall comparison of all experimental parameters and across both sexes it is immediately apparent from the table that the oil-fired power plant fly ash produced more significant changes than coal fly ash (a total of 25 *versus* 20). Generally the same trend could be observed for each individual experimental parameter with the exception of pulmonary free cellular ATP levels. However comparative evaluation of the effect on ATP of the oil fly ash is difficult because of the variability in these data described earlier.

In the case of the pulmonary cellular lavage studies, it is evident from Table 2 and Figure 5 that there are more numerous and greater significant increases in cell counts after exposure to the oil power plant fly ash (8) than to the coal fly ash (5). In contrast, for ATP levels the coal fly ash produced more (7) significant and consistent changes (all decreases), than the oil fly ash (3 increases and 1 decrease were significant).

Table 2 and Figure 6 demonstrate that there is a greater incidence as well as magnitude of effect in the significant increases in mortality due to streptococcus infection after inhalation of the oil power plant fly ash (6) than after inhalation of the coal fly ash (4). In addition two significant decreases in mortality were also caused by exposure to the oil fly ash.

TABLE 2. SUMMARY OF SIGNIFICANT CHANGES IN EXPERIMENTAL PARAMETERS AFTER EXPOSURE TO AEROSOLS OF A COAL-FIRED POWER PLANT FLY ASH OR AN OIL-FIRED POWER PLANT FLY ASH

Sample	Conc, $\mu g/m^3$ Mean	SD	No. of Exposures, 3 hr/day	Total Cells M	F	Cellular ATP M	F	Mortality M	F	Bactericidal Activity M	F
Coal-Fired Power Plant Fly Ash	2028	322	5	+	0	0	-	0	+	+	+
			10	+	+	0	-	0	0	0	0
			20	0	+	0	0	0	+	0	0
	1030	187	5	0	0	-	-	0	0	+	0
			10	+	0	-	-	0	0	0	0
			20	0	0	-	0	+	+	0	+
Oil-Fired Power Plant Fly Ash	1993	222	5	0	0	0	-	+	+	+	-
			10	+	+	0	0	+	+	-	0
			20	+	+	0	+	+	0	0	0
	1053	208	5	0	0	+	0	0	0	0	-
			10	+	+	0	0	+	0	0	0
			20	+	+	+	0	-	-	0	-

+ Significantly greater than control ($p<0.10$)

- Significantly lower than control ($p<0.10$)

0 No significant change from control

M Male

F Female

Significant changes in pulmonary bactericidal activity to *K. pneumoniae* were generally depressions (4 out of 5) for the oil power plant fly ash, whereas only siginficant increases (4) were found after exposure to the coal fly ash.

Comparing the effects of a fluidized-bed coal fly ash and a copper smelter dust in another study (ref. 2),we found the copper smelter dust more cytotoxic to rabbit alveolar macrophages *in vitro* (as mentioned earlier) and producing more detrimental effects in inhalation exposures in mice *in vivo*. Pulmonary bactericidal activity to *K. pneumoniae* showed consistent significant depressions after inhalation of the copper smelter dust, whereas significant changes in bactericidal activity after exposure to the fluidized-bed coal fly ash were all increases. (Under these conditions corresponding mortality rates from streptococcus infection generally demonstrated significant increase and no change, respectively). We also found that the copper smelter dust contained water-soluble components (e.g. arsenic) that contributed to the *in vitro* cytotoxicity of the non-soluble particles.

A recent exploratory *in vitro* examination of the oil power plant fly ash showed similar behavior, i.e., in addition to the particles *per se*, their water-soluble leachate was also cytotoxic to alveolar macrophages *in vitro*. Adding interest to this observation was the subsequent identification of vanadium compounds in the soluble leachate. This correlates well with our findings in an early study (ref. 3) demonstrating extensive functional, biochemical and surface morphological damage in rabbit alveolar macrophages due to *in vitro* exposure to soluble vanadates. In contrast neither of the two coal fly ashes (the fluidized-bed-, or the presently discussed conventional-coal conversion fly ash) produced soluble cytotoxic components and these samples showed only low to intermediate cytotoxicity to rabbit alveolar macrophages *in vitro* (ref. 2).

Thus our studies have consistently demonstrated that aerosols of particulate samples with higher relative *in vitro* cytotoxicity and partial solubility produced significant depression in pulmonary bactericidal activity to inhaled *K. pneumoniae* and increased mortality from experimentally induced streptococcal pneumonia, whereas inhalation of more inert particles (such as the coal fly ashes) could be correlated with bactericidal activity increases and with no change in mortality.

In terms of the present studies,this further confirms the evaluation of the oil power plant fly ash as the relatively greater inhalation hazard than the coal fly ash. To keep this observation in proper perspective it should be remembered however, that the coal fly ash was collected from an electrostatic precipitator,and samples obtained from such in-plant pollution control devices are generally removed from the effluent stream at relatively high temperatures prior to cooling and dilution. The more volatile compounds, which may also be more toxic, are not collected and, therefore, are not present on these particles. The oil-fired power plant fly ash was an actual fugitive emission sample collected from the stack gases and thus the particles probably contained more hazardous trace metals as well as aliphatic and/or aromatic hydrocarbon compounds condensed on their surfaces.

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Copy No.	Recipient
1 - 12	Health Effects Research Laboratory U.S. Environmental Protection Agency Research Triangle Park, NC 27711
1 - 10	Dr. Judith A. Graham, Chief Inhalation Toxicology Branch Environmental Toxicology Division MD-82
11	Dr. Donald E. Gardner, Director Environmental Toxicology Division MD-66
12	Dr. Fred Miller Inhalation Toxicology Branch Environmental Toxicology Division MD-82
13 - 18	IIT Research Institute Main Files Div. L Files C. Aranyi J. Fenters J. Bradof R. Kettlewell

APPENDIX

Additional, more detailed experimental data are provided here for the EPA Project Officer's review. Tables A-1, A-2 and A-3 show results from the pulmonary cellular lavage, susceptibility to infection and bactericidal activity studies respectively summarized in Table 1 and Figures 5 through 7 of the report.

TABLE A-1. EFFECT OF MULTIPLE DAILY 3-HR EXPOSURES TO AEROSOLS OF A COAL-FIRED POWER PLANT FLY ASH OR AN OIL-FIRED POWER PLANT FLY ASH ON THE PULMONARY FREE CELLS LAVAGED FROM THE LUNGS OF CD_1 MICE

Sample	Conc. µg/m³ Mean	SD	Sex	Exposure Conditions Number, 3 hr/day	Treatment Group	% Viability Mean	SE	N(a)	Cell Count Total x 10^5 Mean	SE	N(a)	Macrophage % Mean	SE	N(a)	ATP 10^8fg/10^5 cells Mean	SE	N(a)
Coal-fired Power Plant Fly Ash	2028	322	M	5	c(b)	96.6	0.3	45	6.47	0.44	45	93.0	1.2	29	1.27	0.04	38
					x(c)	96.5	0.3	39	7.30**	0.38	39	92.9	1.2	27	1.19	0.03	38
				10	c	98.3	0.3	10	3.78	0.81	10	96.6	1.5	5	1.48	0.12	8
					x	97.9	0.4	8	7.74**	1.01	8	100. *	-	4	1.35	0.05	8
				20	c	97.3	0.3	24	7.52	0.71	24	94.2	1.2	15	1.59	0.07	24
					x	96.6	0.4	25	8.81	0.70	25	93.8	1.0	16	1.55	0.09	25
	1030	187	M	5	c	98.3	0.2	16	4.39	0.54	16	99.9	0.1	8	1.37	0.08	16
					x	98.2	0.2	17	5.45	0.49	17	99.8	0.2	8	1.17**	0.06	16
				10	c	98.1	0.3	16	3.26	0.34	16	99.4	0.3	8	1.41	0.07	16
					x	98.1	0.3	16	4.88**	0.68	16	99.6	0.2	8	1.10**	0.08	16
				20	c	98.6	0.2	16	4.55	0.32	16	99.6	0.2	8	1.62	0.13	16
					x	98.3	0.2	16	4.28	0.51	16	99.7	0.2	7	1.18**	0.12	16
	2028	322	F	5	c	96.9	0.3	33	5.33	0.34	33	95.9	1.0	12	1.28	0.05	29
					x	97.4	0.2	34	5.14	0.39	34	97.5	0.8	10	1.14**	0.04	27
				10	c	97.9	0.2	14	2.60	0.28	15	98.6	0.4	5	1.83	0.09	8
					x	97.9	0.1	9	3.63*	0.55	8	99.5	0.5	4	1.10***	0.14	8
				20	c	98.3	0.3	9	3.34	0.52	9	99.6	0.4	5	1.17	0.11	7
					x	97.9	0.3	10	4.91*	0.66	10	99.3	0.3	4	1.32	0.09	8
	1030	187	F	5	c	98.5	0.2	16	3.60	0.37	16	98.0	0.5	8	1.17	0.06	16
					x	98.4	0.2	16	3.93	0.36	16	99.4*	0.3	8	0.99**	0.05	14
				10	c	98.1	0.2	27	3.43	0.19	27	98.3	0.5	12	1.32	0.05	24
					x	97.9	0.2	24	3.81	0.29	24	99.4*	0.2	13	1.08**	0.07	23
				20	c	97.9	0.2	36	4.34	0.36	36	98.8	0.3	16	1.33	0.05	33
					x	97.9	0.2	38	4.54	0.37	38	98.4	0.4	17	1.32	0.05	35

TABLE A-1 (continued)

Sample	Conc. μg/m³ Mean	Conc. μg/m³ SD	Sex	Exposure Conditions: Number, 3 hr/day	Exposure Conditions: Treatment Group	% Viability: Mean	% Viability: SE	% Viability: N(a)	Cell Count: Total x 10⁵ Mean	Cell Count: Total x 10⁵ SE	Cell Count: Total x 10⁵ N(a)	Cell Count: Macrophage % Mean	Cell Count: Macrophage % SE	Cell Count: Macrophage % N(a)	ATP: 10⁸fg/10⁵ cells Mean	ATP: 10⁸fg/10⁵ cells SE	ATP: 10⁸fg/10⁵ cells N(a)
Oil-fired Power Plant Fly Ash	1993	222	M	5	c	98.8	0.1	16	3.77	0.44	15	99.6	0.2	8	1.43	0.09	16
					x	98.7	0.2	16	4.03	0.39	16	99.9	0.1	8	1.39	0.07	15
				10	c	98.6	0.2	16	3.03	0.20	16	99.6	0.3	8	1.38	0.05	16
					x	98.6	0.2	16	6.78***	0.44	16	99.9	0.4	8	1.46	0.09	16
				20	c	98.6	0.2	16	4.95	0.32	16	99.6	0.2	8	1.62	0.14	16
					x	98.9	0.1	16	7.53***	0.67	16	99.1	0.5	8	1.52	0.10	16
	1053	208	M	5	c	98.4	0.2	17	3.74	0.49	19	96.3	1.5	8	1.20	0.07	17
					x	98.8*	0.1	19	3.55	0.24	17	97.0	1.6	8	1.39*	0.07	16
				10	c	98.6	0.1	18	3.57	0.38	18	98.3	0.7	8	1.36	0.05	18
					x	98.7	0.2	16	4.80**	0.68	16	97.8	0.7	8	1.39	0.10	14
				20	c	98.5	0.2	16	3.92	0.33	16	99.4	0.3	8	1.32	0.09	15
					x	98.7	0.2	16	5.50**	0.49	16	99.3	0.4	8	1.69**	0.07	16
	1993	222	F	5	c	98.5	0.2	16	3.38	0.32	16	98.5	0.6	8	1.42	0.07	16
					x	98.8	0.1	16	3.52	0.28	16	99.0	0.2	8	1.21**	0.04	15
				10	c	98.3	0.2	17	3.33	0.30	17	97.5	0.8	8	1.34	0.06	16
					x	98.1	0.3	17	5.93***	0.37	18	99.3*	0.4	8	1.28	0.08	15
				20	c	98.4	0.2	17	3.44	0.35	17	98.8	0.6	8	1.33	0.08	16
					x	98.2	0.3	16	6.14***	0.50	16	99.0	0.5	8	1.57**	0.06	15
	1053	208	F	5	c	98.6	0.2	17	2.89	0.19	17	95.4	2.7	10	1.11	0.06	16
					x	98.5	0.2	17	3.31	0.25	17	89.1	3.8	12	1.24	0.09	17
				10	c	98.6	0.2	19	3.69	0.31	19	99.5	0.3	8	1.22	0.08	15
					x	98.8	0.1	18	5.63***	0.42	18	99.9	0.1	8	1.09	0.08	15
				20	c	98.8	0.2	16	3.28	0.31	16	99.9	0.1	8	1.63	0.09	16
					x	98.5	0.2	18	5.79***	0.42	18	99.0*	0.4	8	1.56	0.10	17

(a) number of mice

(b) control mice exposed to filtered air

(c) experimental mice

Significant change from corresponding control mice: $p \leq 0.1$ **$p \leq 0.05$ ***$p \leq 0.001$

TABLE A-2. EFFECT OF MULTIPLE DAILY 3-HR EXPOSURES TO AEROSOLS OF A COAL-FIRED POWER PLANT FLY ASH OR AN OIL-FIRED POWER PLANT FLY ASH ON MORTALITY RATES AND MEAN SURVIVAL TIME AFTER RESPIRATORY *STREPTOCOCCUS* CHALLENGE IN CD_1 MICE

Sample	Conc. $\mu g/m^3$ Mean	± SD	Sex	Exposure Conditions: Number, 3 hr/day	Treatment Group	Mortality: D/T	%	% Change	Mean Survival Time: Day	% Change
Coal-fired Power Plant Fly Ash	2028	322	M	5	c[a]	32/191	16.8	0	13.2	0
					x[b]	32/191	16.8		13.2	
				10	c	59/197	29.9	-0.1	11.8	0.1
					x	59/198	29.8		11.9	
				20	c	36/117	30.8	8.4	11.5	-0.9
					x	47/120	39.2		10.6	
	1030	187	M	5	c	40/157	25.5	3.7	11.7	-0.4
					x	47/161	29.2		11.3	
				10	c	34/132	25.8	-2.1	11.7	0.4
					x	32/135	23.7		12.1	
				20	c	7/150	4.7	4.9*	13.7	-0.4*
					x	14/146	9.6*		13.3*	
	2028	322	F	5	c	19/238	8.0	5.9**	13.4	-0.2
					x	33/237	13.9**		13.2	
				10	c	58/193	30.1	5.3	12.0	-0.6
					x	68/192	35.4		11.4	
				20	c	10/69	14.5	17.9**	12.9	-1.2
					x	22/68	32.4**		11.7**	
	1030	187	F	5	c	10/145	6.9	1.1	13.5	-0.1
					x	12/150	8.0		13.4	
				10	c	12/206	5.8	0.1	13.5	0.1
					x	10/169	5.9		13.6	
				20	c	29/155	24.6	4.5**	12.6	-0.6*
					x	52/179	29.1**		12.0*	
Oil-fired Power Plant Fly Ash	1993	222	M	5	c	14/137	10.2	18.2***	13.2	-1.6***
					x	40/141	28.4***		11.6***	
				10	c	7/113	6.2	18.8***	13.5	-1.5***
					x	29/116	25.0***		12.0***	
				20	c	7/150	4.7	10.7**	13.7	-0.9***
					x	24/156	15.4**		12.8***	
	1053	208	M	5	c	18/119	15.1	-0.7	12.9	-0.1
					x	17/118	14.4		12.8	
				10	c	2/78	2.6	9.3**	13.8	-0.8**
					x	8/67	11.9**		13.0**	
				20	c	11/140	7.9	-5.6**	13.5	0.4**
					x	3/133	2.3**		13.9**	
	1993	222	F	5	c	11/161	6.8	25.2***	13.5	-2.5***
					x	54/169	32.0***		11.0***	
				10	c	9/161	5.6	5.1*	13.6	-0.5*
					x	16/149	10.7*		13.1*	
				20	c	2/72	2.8	2.6	13.9	-0.4
					x	4/74	5.4		13.5	
	1053	208	F	5	c	9/147	6.1	1.9	13.5	-0.2
					x	12/150	8.0		13.3	
				10	c	9/119	7.6	-0.1	13.4	0
					x	9/120	7.5		13.4	
				20	c	18/106	17.0	-12.3**	13.0	0.7**
					x	5/106	4.7**		13.7**	

(a) control mice exposed to filtered air

(b) experimental mice

Significant change from corresponding infected control mice: *$p \leq 0.1$ **$p \leq 0.05$ ***$p \leq 0.001$

TABLE A-3. EFFECT OF MULTIPLE DAILY 3-HR EXPOSURES TO AEROSOLS OF A COAL-FIRED POWER PLANT FLY ASH OR AN OIL-FIRED POWER PLANT FLY ASH ON THE PULMONARY BACTERICIDAL ACTIVITY TO ^{35}S-*K. PNEUMONIAE* IN CD$_1$ MICE

Sample	Conc. µg/m^3 Mean	± SD	Sex	Exposure Conditions: Number, 3 hr/day	Treatment Group	Pulmonary Bactericidal Activity to inhaled ^{35}S-*K. pneumoniae*: % Killed in 3-hr. Mean	SE	N (a)	% Change $x^{(b)}-c^{(c)}$
Coal-fired Power Plant Fly Ash	2028	322	M	5	c	86.8	1.7	24	6.6**
					x	93.4**	1.1	23	
				10	c	85.3	1.0	22	-2.0
					x	83.3	1.4	23	
				20	c	89.4	1.0	12	2.1
					x	91.5	1.4	11	
	1030	187	M	5	c	85.3	1.2	22	2.7*
					x	88.0*	0.9	23	
				10	c	89.3	1.5	20	0.4
					x	89.7	0.9	20	
				20	c	88.1	2.3	19	-3.1
					x	85.0	2.4	17	
	2028	322	F	5	c	86.2	1.3	31	3.6*
					x	89.8*	1.3	27	
				10	c	88.2	1.4	21	1.7
					x	89.9	1.4	23	
				20	c	88.4	1.6	12	1.4
					x	89.8	1.3	12	
	1030	187	F	5	c	93.1	1.0	21	-2.3
					x	90.8	0.8	22	
				10	c	91.0	0.8	45	-0.9
					x	90.1	0.4	41	
				20	c	82.5	1.6	30	6.7***
					x	89.2***	1.1	31	
Oil-fired Power Plant Fly Ash	1993	222	M	5	c	85.3	1.4	21	2.7*
					x	88.0*	0.7	22	
				10	c	90.9	1.0	23	-4.0**
					x	86.9**	1.6	15	
				20	c	88.0	2.3	19	3.8
					x	91.8	0.8	21	
	1053	208	M	5	c	87.0	1.5	21	1.1
					x	88.1	1.3	23	
				10	c	91.5	1.0	22	-2.4
					x	89.1	0.9	23	
				20	c	86.9	2.1	22	0.8
					x	87.7	1.5	22	
	1993	222	F	5	c	92.7	1.2	20	-5.4**
					x	87.3**	1.6	23	
				10	c	89.5	1.3	24	-2.1
					x	87.4	1.5	20	
				20	c	69.9	4.6	21	1.7
					x	71.6	3.1	23	
	1053	208	F	5	c	90.5	1.0	24	-2.5*
					x	88.0*	1.6	22	
				10	c	91.5	1.2	22	1.5
					x	93.0	1.1	21	
				20	c	87.4	1.3	22	-3.5*
					x	83.9*	1.5	23	

(a) number of mice

(b) experimental mice

(c) control mice exposed to filtered air

Significant change from corresponding control mice: *$p \leq 0.1$ **$p \leq 0.05$ ***$p \leq 0.001$

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Copy No.	Recipient
1 - 12	Health Effects Research Laboratory U.S. Environmental Protection Agency Research Triangle Park, NC 27711
1 - 10	Dr. Judith A. Graham, Chief Inhalation Toxicology Branch Environmental Toxicology Division MD-82
11	Dr. Donald E. Gardner, Director Environmental Toxicology Division MD-66
12	Dr. Fred Miller Inhalation Toxicology Branch Environmental Toxicology Division MD-82
13 - 18	IIT Research Institute Main Files Div. L Files C. Aranyi J. Fenters J. Bradof R. Kettlewell